Supporting Information

Synthesis and Characterization of the Oxidized dGTP Lesions Spiroiminodihydantoin-2’-deoxynucleoside-5’-triphosphate and Guanidinohydantoin-2’-deoxynucleoside-5’-triphosphate

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**31P NMR and HMBC NMR Procedure for dSpTP, SpOAc and GhOAc.**

The size exclusion column purified dSpTP (0.2 mg) was dissolved in H2O, while purified SpOAc (1 mg) and GhOAc (1 mg) were dissolved in DMSO-d6. Proton decoupled 31P NMR and HMBC NMR experiments were recorded on each sample with a 500 MHz spectrometer. All spectral data were collected at a temperature of 22°C. TMS and 85% phosphoric acid were used as references.

**ESI-MS Analysis of Nucleotides.**

Nucleotide samples were analyzed by negative electrospray ionization (ESI) equipped with an API source. Samples were dissolved in water and acetonitrile and introduced via infusion at a flow rate of 5 μL min⁻¹. The source and desolvation temperatures were 100 °C and 150 °C respectively. The capillary voltage was set to 4.25 kV, sampling cone voltage to 45 V, and the extractor cone to 3V.

**LC-ESI/MS Analysis of Nucleotides.**

The nucleotide samples were analyzed by negative electrospray ionization (ESI) equipped with an API source Chromatographic separation was accomplished using a C-18 Zorbax (5 μm, 150X1.0 mm) reversed phase column and a linear gradient of 5% solvent B to 30% solvent B over 20 min. Solvent A consisted of 0.05 mM tetrabutylammonium acetate (pH 7.2), while solvent B acetonitrile. The flow rate was 0.1 mL min⁻¹, and UV spectra were recorded at 220 nm. The source and desolvation temperatures were 110 °C and 200 °C, respectively. The capillary voltage was set to 3.5 kV, sampling cone voltage to 38 V, and the extractor cone to 3V.
ESI-MS/MS Analysis of dSpTP.

The dSpTP sample was analyzed by positive ion electrospray ionization (ESI) equipped with an API source. Samples were dissolved in water and acetonitrile and introduced via infusion at a flow rate of 5 µL min⁻¹. The source and desolvation temperatures were 80°C and 120°C, respectively. The capillary voltage was set to 3.1 kV, sampling cone voltage to 48 V, and the extractor cone to 3V. The collision energy was set to 14 eV. Argon, used as a collision for CID experiments, was adjusted to a pressure of 1.7x10⁻⁴ mBar. The mass for the nucleoside base was set in the first scanning analyzer (MS-1) and the precursor ion was subjected to CID in the static quadrupole and the resulting spectrum of the products recorded by scanning the second scanning analyzer (MS-2) between 50 and 200 Daltons. The scan duration and interscan delay were 1.0 and 0.1 seconds, respectively.
Figure S1. Proton NMR Spectra of GhOAc Nucleoside
Figure S2. HMBC NMR Spectra of GhOAc Nucleoside
Figure S3. Expanded Region of Proton NMR Spectra of SpOAc Nucleoside
Figure S4. Expanded Region of HMBC NMR Spectra of SpOAc Nucleoside
Figure S5. $^{31}$P NMR of dSpTP in H$_2$O
Figure S6. Expanded Region of $^{31}\text{P}$ NMR of dSpTP in H$_2$O

$\alpha^{31}\text{P}$

$\beta^{31}\text{P}$

$\gamma^{31}\text{P}$
Figure S7. Negative Ion LC-ESI/MS Analysis of dSpTP.

05Q891 Sm (Mn, 2x3) 1: Scan ES-

m/z = 378
[\textit{dSpMPH}]^-

m/z = 480
[\textit{dSpDPHNa}]^-

m/z = 458
[\textit{dSpDPH}_2]^-

m/z = 560
[\textit{dSpTPH}_2\textit{Na}]^-

m/z = 538
[\textit{dSpTPH}_3]^-

UV-vis
220 nm

5.00 10.00 15.00 20.00 25.00

05Q891 Sm (Mn, 2x3) 2: Diode Array

m/z = 378
4.83e6

m/z = 480
1.91e5

m/z = 458
1.34e6

m/z = 560
3.41e4

m/z = 538
4.44e4

20.95
18.24
Figure S8. HPLC Analysis of Purified dGhTP.
Figure S9. Negative Ion LC-ESI/MS Analysis of Crude dGhTP.
Figure S10. Negative Ion ESI-MS Parent Ion Scan of $[\text{dSpDH}_2]$ (mz = 458).
Figure S11. Positive Ion ESI-MS/MS Analysis of the Free Base of dSpTP.